

Annex 1

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PATENTS 2-14-02

Attorney's Docket No. 3339-239A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Joël Sternheimer
Appl. No.: To Be Assigned
Filed: Concurrently Herewith
For: METHOD FOR THE REGULATION OF
PROTEIN BIOSYNTHESIS

May 25, 1999

Assistant Commissioner for Patents
Washington, DC 20231

**RULE 132 DECLARATION
OF JOËL STERNHEIMER**

Sir:

I, JOËL STERNHEIMER, do hereby declare and say as follows:

1. That I am a graduate of Paris, Lyons and Princeton Universities and received my degrees in the years 1964, 1966 and 1967 (Doctorate in 1966).
My Curriculum Vitae and the list of publications are attached thereto (Annexes 1 and 2).
2. That it clearly emerges from the Invention as now defined that it does not lack utility: the method of the invention allows and controls the *in situ* regulation of the synthesis of selected protein. It was undertaken by Mr. Pedro Ferrandiz under my supervision to stimulate the growth of blue-green algae —prokaryotes, genus *Anabaena*— by epigenetic regulation. Their photosynthetic activity involves in particular pigmentary proteins (cyanins). Thus their biosynthesis is easily observed through color change and oxygen release.

Consistent
4/14/02
[Signature]

We want to point out that this first attempt of stimulation in an aquatic medium is relatively simple to reproduce. We believe that the results obtained are particularly promising. One may add the fact that it points towards numerous applications.

- Materials and methods

- Dilution of 12 ml of *Anabaena variabilis* (stock provided by the Ecole Normale Supérieure de Paris) in 1 500 ml of mineral water.
- Addition of 40 g of dry vegetable manure containing 8%, say 2.6 g, of nitrates as well as 40 g of river pebbles (as suggested by Vincent Bargoin this would provide the solution with trace elements).
- Adaptation time to the cultures medium: four days.
- Transfer of 750 ml of the solution in two vats subjected to natural enlightenment. This setting in culture started on the 30th of April.

Musical diffusions.

The music has been diffused in one of the vats, by mean of an aquatic speaker Altec UW-30, while the other vat served as a control.

The proteins transcribed in musical sequences were the following:

- TAPE I (45 min)
 - NIF H of *Anabaena v.* (five times)
 - Allophycocyanin of *Anabaena v.* (three times)
 - Plastocyanin of *Anabaena v.* (three times)
 - Nitrate reductase of *Chorella s.* (three times)
 - PS1 photosystem protein of *Anabaena v.* (three times) (*)
 - Ferredoxin of *Anabaena v.* (five times)
 - Protein 35 K of *Anabaena v.* (eight times) (*)
- TAPE II (15 min)
 - Allophycocyanin of *Anabaena v.* (two times)
 - Plastocyanin of *Anabaena v.* (two times)
 - PS1 photosystem protein of *Anabaena v.* (three times) (*)
 - Ferredoxin of *Anabaena v.* (four times)
 - Protein 35 K of *Anabaena v.* (eight times).
- TAPE III (15 min)
 - Ferredoxin of *Anabaena v.* (two times)
 - NIF H of *Anabaena v.* (three times)

NIF A of Anabeana v. (three times) (*)

NIF D of Anabeana v. (three times) (*)

Nitrate reductase of Chlorella s. (three times)

Protein 35 K of Anabeana v. (two times) (*).

The transcriptions had been realized by J. Sternheimer on a sampler Casio SK1 apart from those labelled (*) which were made by P. Ferrandiz on a « One Key Play » software written by Sylvie Guillou and Fabrice Ocelli (INSERM St-Anne, Paris). The rate of the transpositions is tuned so as to make their length correspond to the photoperiods of the micro-organisms.

Tape I was played twice a day, from the 30th of April to the 5th of May. Then from the 7th to the 10th of May TAPE II was played in the morning while TAPE III was played in the evening.

During this period the viability of the micro-organisms was regularly controlled: Samples were drawn from the cultures and then checked under a microscope.

• Results (Annex 3)

- Evolution of the coloration of cultures (Figure 1)

One poured in the vats the solutions looked opaque (after tossing).

This was due to the manure mentioned above, the dilution rate of the original stock but also to the spread of a fibrous contaminant which was not characterized.

From the 2nd day of listening the musical vat presented a greater proportion of suspending matter than the control one. However this trend reversed itself by the 4th day. We therefore assumed that the musical exposures had been too long and we decided to abort the diffusion of Tape I.

Instead Tapes II and III have been used. We then observed on May 8th that the tint of the cultures in the musical vat displayed a green blue coloration more pronounced than those in the control vat (Figure 2).

This trend kept increasing up to the end of the experiment.

- Oxygen release.

Ten days after the end of the period of diffusion the musical cultures became characterized by a proliferation of bubbles at the surface (Figure 3).

Since these bubbles had the property to revive the flame of a lighted match which was put close by, we concluded they contained oxygen. On May 24th there were about 70 surface bubbles and on the 28th they were 130 (Figure 4). We point out that the maximum number of visible bubbles observed in the control vat is 8. Hence there is more than a factor 16 between the two cultures with respect to oxygen release. In fact the medium of the musical culture was saturated with oxygen at the end of the observation time. Clearly this is correlated to an increase of the photosynthetic activity in the musical vat. It indicates that while the oxygen was released some carbonated composites have been fixed (Figure 5, taken six months later). Thus this particular application of the epigenetic regulation process led to an interesting depollutive system. This should beget further interests.

Other experiences showing the utility of the instant invention are herewith attached (Annexes 4-8).

As regards a garden experience: See Annex 9.

Figure 1 of this Annex 9 also attached is a comparative test:

On the left side (control): non treated tomatoes

On the right side: tomatoes having received during 16 days 3 minutes per day, the music of protein of anti-drought protein TAS14. Both control tomatoes and the treated tomatoes having 1 1/2 litres of water per plant per day.

3. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

4. Further declarant saith not.

Joël Sternheimer *JS*
Joël STERNHEIMER

1999/5/19
Date

CURRICULUM VITAE of Joël Sternheimer

Director, Collège des Sciences, European University of Research, 1 rue Descartes, 75005 Paris (France); phone/fax 01 46 34 36 78 - Home address: 46, rue de la Montagne Ste Geneviève 75005 Paris; phone/fax 01 43 25 80 11 - e-mail JMSternhei@aol.com - Website <http://members.aol.com/JMSternhei> - Born on January 31st, 1943 at Montluel (Ain, France); married on September 6, 1991 at Seix (Ariège, France) with Yuki, born Yamaguchi.

- * Doctor in Theoretical Physics (3rd cycle) (Lyons, 1966).
- * Diplôme d'Etudes Supérieures de Mathématiques approfondies (\approx M. Sc. Mathematics) (Paris, 1964).
- * Licencié ès sciences mathématiques (\approx B. Sc. Mathematics) (Paris, 1964).
- * "Ettore Majorana scholarship" Laureate 1966 (Erice, Sicily).
- * Graduate alumnus *67 of Princeton University (U. S. A.).
- * Holder of patents n° 83 02122 (France 10/2/83, granted 19/9/86) and n° 92 06765 (France 4/6/92, granted 13/7/95, as well as in 16 other countries including AIPO, Australia and Russia).
- * Philips Science Prize Laureate, 1999.

Until 1967: student and researcher at Lyons, Paris and Princeton universities; publications, doctorate on elementary particles.

1967 to 1971: following U. S. funds shortcuts due to the Vietnam war, I decided to pursue my theoretical physics research (for which Louis de Broglie had sent me to Princeton) as an independent researcher; then living mainly from author and composer royalties (admitted to the Society of Music Authors, Composers and Editors, 1967 and to the Society of Dramatic Authors and Composers, 1968): records, concerts, plays, movies..

1971 to 1974: teaching of my researches' first results at Vincennes (University Paris-VIII), Jerusalem (Hebrew University & Van Leer Foundation) and Paris (E.S.C.P.).

1974 to 1978: author and composer (records, concerts...), and pursuing of the independent research work.

1978 to 1984: full-time independent researcher: publications, patent, seminars (Collège de France...).

1984: scientific counselor at the Cité des Sciences et de l'Industrie (Paris), with patent license conceded.

1986 to 1988: director of seminars at the Collège International de Philosophie (Paris); co-organizer of the Colloquium "Louis de Broglie, physicien et penseur" on the Montagne Ste-Geneviève site (former Ecole Polytechnique); et development of the work as independant researcher.

1988-1999: full-time independent researcher; patent filed in June 1992, publications. Since December 1994: director of seminar at the European University of Research; 1999: awarded Philips Science Prize; director of EUR's Collège des Sciences; President and founder of the Réseau associatif de Chercheurs indépendants.

Scientific papers by Joël Sternheimer

Sur les formules de masse fortes et électromagnétiques des particules élémentaires (avec M. Flato), Comptes Rendus de l'Académie des Sciences (Paris), vol. 259, p. 3455 (1964).

On the masses of nonstrange pseudoscalar mesons and the generalized Klein-Gordon equation (with M. Flato, D. Sternheimer, J. P. Vigiér et G. Wataghin), Nuovo Cimento 42, p. 431 (1966).

Sur les formules de masse des particules élémentaires, Thesis n° 186 (Lyons, 1966).

Strong and weak interactions: present problems (discussions with S. Coleman, M. Gell-Mann, S. L. Glashow), 1966 international school of physics "Ettore Majorana", ed. A. Zichichi, Academic Press 1966, pp. 731 et suivantes.

Théorie des systèmes hiérarchiques, cours donné à Paris-VIII Vincennes en 1971-72, ronéotypé dans le cadre des activités des groupes expérimentaux de l'université Paris-VII, 1971.

Musique des particules élémentaires, Comptes Rendus de l'Académie des Sciences (Paris), vol. 297, p. 829 (1983).

Musique des particules élémentaires: invariance d'échelle, quantification et lois musicales dans la matière, mathematical physics seminar (A. Lichnerowicz), Collège de France, 1984; reproduced in Revue de Bio-Mathématique, vol. 94, p. 1 (1986).

Procédé de modélisation acoustique des particules élémentaires, Brevet français n° 83 02122 (n° de publication 2 541 024).

Instrument à cordes pour la modélisation acoustique des particules élémentaires (avec P. Fléjo, J. P. Favino et J. C. Trébuchet), Certificat d'addition n° 84 08292 (n° de publication 2 565 016).

Musique des particules élémentaires, le Cahier du Collège international de philosophie n° 3, 180 (1987).

Intervention au Colloque International "Louis de Broglie, physicien et penseur", Ancienne Ecole Polytechnique, Paris 1987 (à paraître).

Procédé de régulation épigénétique de la biosynthèse des protéines par résonance d'échelle, french patent n° 92 06765 (publication n° 2 691 976, granted 13.07.95), european n° 93 913 082.9 (publication n° 0 648 275), Israel n° 105855, Tunisia n° SN.93.063 (holder V. Bargoin, granted under n° 16704), PCT n° PCT/FR 93/00524 (publication n° WO 93/24645, 12/9/93), including AIPO (13 countries) n° 60587 (granted 12.18.96, under n° 10113), Australia n° 48848/93 (granted 10.16.97, under n° 679181), Japan n° 6-500268, (publication n° 7-507287), Corea n° 94-704444, Canada n° 2,136,737, Russia n° 94046307.00 (granted 12.8.97, under n° RU 2113487), U.S.A. n° S.N. 08/347.353.

Ondes d'échelle: une expression spécifique de la covariance des lois physiques lors d'un changement d'échelle d'observation, Note présentée à l'Académie des Sciences le 3/10/97 par André Lichnerowicz. *Ondes d'échelle*, 1992 (non publié); letter to New Scientist n° 1937, p. 50 (1994); divers Rapports et Comptes-rendus d'expériences, in responses to EPO Official Notifications, accessible through Internet <http://members.aol.com/JMSternhei>. A brief account can be found in J.M.Pelt's book, *Les langages secrets de la nature*, chapitre 18, rééd. Le Livre de Poche 1998; a more detailed in Y. Fukagawa's book to appear in Japan (sept. 99).